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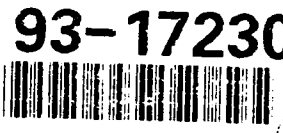

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13. ABSTRACT (Maximum 200 words)

The physiological effect known as long-term potentiation (LTP) is widely suspected of being the substrate of several forms of memory encoded by synapses in the forebrain of humans and other mammals. Work in the past year established that translational suppression with antisense oligonucleotides of one of the subunits of the AMPA (glutamate) transmitter receptor blocks the capacity of synapses to exhibit LTP. This confirms the hypothesis that the AMPA receptor is the agent of LTP expression. Changes in the waveform of the synaptic responses were found to occur in conjunction with LTP and were suggestive of a shift in the kinetic properties of the AMPA receptor channel. Computer simulations of the receptor led to the discovery that all known phenomenology of LTP expression can be reproduced by simply increasing the rates at which the receptor channel opens and closes. In parallel studies, a drug which acts on the AMPA receptor channel was shown to facilitate the induction of LTP. This led to a drug development program to find potent compounds of this kind which cross the blood-brain barrier and enhance the AMPA receptor and LTP in behaving rats. This effort was successful and one of the new drugs was then tested extensively in large numbers of rats across three learning tasks; as predicted, this compound produced substantial improvements in the encoding of short and long-term memories.

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The goals of this research program are as follows: i) identify the cellular mechanisms responsible for inducing, expressing, and stabilizing long-term potentiation (LTP) and ii) analyze the contributions of LTP to behavioral memory. Substantial progress has been made in the past year in realizing these objectives.

Role of AMPA (glutamate) receptors in expression of LTP

Work described in past Progress Reports led by negative evidence to the conclusion that changes in post-synaptic glutamate receptors are responsible for the enhanced transmission that constitutes LTP. Positive evidence that induction of LTP alters receptors was then obtained: first, aniracetam, a drug which modulates the AMPA receptor channel, was found to have different effects on potentiated vs. control synapses; second, it was discovered that LTP alters the decay time constant of synaptic responses, as expected if it modifies channel kinetics (see 1992 Progress Report). Three developments during the past year have confirmed and extended these first results and have led to a specific hypothesis regarding the nature of the receptor modification underlying LTP.

- *Changing the subunit composition of the AMPA receptor eliminates expression of LTP*

  The AMPA receptor, which mediates fast excitatory transmission in telencephalic networks, is composed of at least three homologous proteins. During the past year, we showed that modifying the balance of the subunits drastically affects LTP. The studies were carried out using a recently introduced technique for maintaining long-term cultures of hippocampus. Antisense oligonucleotides directed at the mRNA encoding one of the receptor subunits (GluR-1) were constructed, suspended in a lipofectant, and applied to the cultures for ten days. Sense probes were used as controls. Successful transfection was achieved as indicated by a marked drop in the concentration of GluR-1 protein (assayed with antibodies prepared against an amino acid sequence specific to the subunit) without any change in the concentration of GluR-2/3 subunits. Synaptic responses were not detectably changed following this treatment, as anticipated from earlier observations by Heinneman and co-workers that GluR-2 and 3 alone combine to form functional receptors. LTP, however, was virtually absent in the transfected slices (Vanderklish *et al.*, 1992; copy attached). Work is now in progress to determine if the GluR-1 plays a special

role in LTP or if any change in balance of receptor subunits eliminates the capacity of the composite receptor to express the potentiation effect

- *LTP changes the entire waveform of the synaptic response*

  If LTP modifies channel kinetics, then it should affect all phases of the synaptic response and not simply the decay time constant. This was confirmed using *in vitro* slices in which fast and slow inhibitory currents and cell spiking were suppressed. These experiments showed that LTP distorts the entire waveform of the response and that these distortions can be eliminated by stretching the response by the same percentage as the change in the decay time constant; i.e., LTP results in a uniform contraction of the waveform. Moreover, the LTP induced reduction in the decay time constant was also obtained in slices from immature rats which have poorly developed dendrites and spines. This indicates that the effect is independent of spine biophysics. Increasing the size of the response by means other than LTP produced none of the waveform distortions obtained with the potentiation effect. Together these results provide strong evidence that LTP is associated with a modification in the rate constants governing the behavior of the receptor channel (Ambros-Ingerson *et al.*, in press).

- *A formal hypothesis regarding the receptor changes responsible for LTP*

  A computer simulation of the AMPA receptor was developed using published estimates of rate parameters and found to reproduce essential functional characteristics of the receptor. This work led to the discovery that simply increasing the opening/closing rates of the receptor channel causes an LTP-like increase in the slope and amplitude of the synaptic current; prior to this we had assumed that a change in the conductance of the channel was necessary to produce an increase in the size of the synaptic response. Instead it appears that a shift in the open/close rates sufficient to reproduce the waveform distortion associated with LTP also reproduces the percent increase in slope and amplitude found with potentiation. The effects of aniracetam could be obtained with the model by slowing desensitization kinetics; combining this with kinetic changes in the open/close rates resulted in an interaction very much like that found between aniracetam and LTP in physiological experiments. Thus, we have arrived at the very specific hypothesis that LTP is due to an acceleration of the opening and closing of the AMPA receptor channel (Ambros-Ingerson *et al.*, 1993b).

LTP and Memory

Results from pharmacological experiments constitute one line of evidence implicating LTP in memory. Studies from this laboratory and elsewhere have shown that inhibitors of LTP cause behavioral amnesia (see previous Progress Reports) and we have recently found that one class of drugs known to impair memory encoding in animals and humans also disrupts the induction of LTP (del Cerro *et al.*, 1992). In the past year we also published experiments showing that drugs which enhance synaptic transmission or which block long hyperpolarizing potentials promote the induction of LTP and/or raise the ceiling on the maximal degree of LTP (Arai *et al.*, 1993a). This led to a general hypothesis about the local circuit events and receptors which control the amount of afferent activity needed to induce potentiation; confirmation of certain key predictions of the hypothesis was obtained in a study of LTP in the basal dendrites of hippocampal pyramidal neurons (Arai *et al.*, 1993b). These advances in our understanding of the factors controlling LTP induction (and reversal) led to efforts at designing drugs which would promote the potentiation effect in behaving animals. The overall goal of this was to test if facilitation of LTP will enhance the encoding of memory. Aniracetam, as noted, prolongs the open time of the AMPA receptor and in this way facilitates excitatory transmission (see previous Progress Reports). It promotes LTP induction in slices of hippocampus and thus was used as a lead in developing centrally active compounds. Variants of the drug which lack the bond at which aniracetam is metabolized in the periphery were developed and screened for their effects on synaptic transmission first *in vitro* and then *in vivo*. Four drugs have now been developed which cross the blood-brain barrier after interperitoneal injections and produce aniracetam-like effects on synaptic transmission in freely moving rats. One member of this group has also been extensively tested for its actions on behavioral memory. The results were striking: the analogue produced a marked reduction in the number of trials needed to form stable memory in a two odor discrimination problem and in a water maze task involving spatial cues. It also prolonged memory in a radial maze paradigm. These effects were sizeable (e.g., the analogue reduced by 50% the number of memory errors in the radial maze) and highly significant (Staubli *et al.*, submitted). Thus, the work on LTP mechanisms has led to a novel, biologically based approach to memory enhancement.

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